

Learning and Memory

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Abstract Learning and memory processes are thought to underlie a variety of human psychiatric disorders, including generalised anxiety disorder and post-traumatic stress disorder. Basic research performed in laboratory animals may help to elucidate the aetiology of the respective diseases. This chapter gives a short introduction into theoretical and practical aspects of animal experiments aimed at investigating acquisition, consolidation and extinction of aversive memories. It describes the behavioural paradigms most commonly used as well as neuroanatomical, cellular and molecular correlates of aversive memories. Finally, it discusses clinical implications of the results obtained in animal experiments in respect to the development of novel pharmacotherapeutic strategies for the treatment of human patients.

Keywords Learning · Memory · Fear · Anxiety · Conditioning · Reconsolidation · Extinction · Sensitisation · PTSD

1

Introduction

One common characteristic of animals throughout the animal kingdom is their ability to adapt to suddenly changed environmental conditions. If these adaptations rest on modifications of the nervous system and become evident at the behavioural level, we say that the animals have learned. Whereas learning describes the process of adaptation, memory refers to the state and persistence of the adaptive behavioural changes. A typical learning curve consists of several subsequent phases. Memory acquisition is the phase of acute interaction of an organism with its changing environment that is characterised by admission of sensory information. During memory consolidation, the acquired information is further processed within the brain, leading to transient or lasting changes in interneuronal communication, i.e. to formation of memory engrams. Animals show memory retention as long as they are principally able to retain the adaptive changes of their behavioural performance. This, however, does not mean that they are always able to retrieve/recall the memory, i.e. to 'translate' the altered interneuronal communication into the adaptive behavioural changes. Most memories dissipate with time, due to reversal of the original changes in interneuronal communication or to additional modification of the respective neuronal circuits by new learning processes that disturb retrieval of the original memory. Memories can also be actively extinguished by training. The resulting state of reduced memory performance is called retention of memory extinction and is thought to rest on the formation of new memories that counteract the retrieval of the original one. The existence of various phases of memory processing underscores the importance of clearly describing which stage of the learning curve is targeted by a pharmacological study.

With the advent of molecular biology, refinements of neurophysiological tools and selection of suitable animal models, it became more and more feasible to search for the cellular basis of memory. This chapter will briefly summarise the results of this search together with a description of the methods that led to the discoveries. In this context, I will largely concentrate on aversively motivated learning and memory that enable us to recognise and to appropriately respond to potentially dangerous situations. These abilities, which ensured the survival of man and animal throughout evolution, bear the risk of pathological alteration that might be directly linked to distinct human anxiety disorder, such as phobias or post-traumatic stress disorder. In the beginning of this chapter, I will briefly introduce behavioural paradigms and animal models that turned out to be useful for the study of aversive memories, followed by a short description of neurological substrates and cellular mechanisms that

are involved in the respective memory processes. I will end with a discussion of how closely animal experiments resemble the situation in human beings.

This chapter has been written for pharmacologists and physicians interested in the ways of studying the involvement of learning and memory in the aetiology of pathological anxiety. Hopefully, it will provide a guideline for better understanding the rationales and experimental strategies of the respective animal experiments and stimulate the searching for novel therapeutic targets. At the same time, it should sharpen the attention for potential caveats of various methodological approaches (cf. Wotjak 2004). It is not my intention to give an exhaustive review on the formation and extinction of aversive memories and the clinical impact of these processes. Readers interested in more detailed information will be referred to more specialised publications.

2

Behavioural Paradigms for Studying Aversive Memories

Motivation is essential for both memory acquisition/consolidation and memory retrieval. Principally, animals are motivated to approach rewarding and to avoid aversive situations. This has been used for the development of behavioural paradigms that help to study memory processing of appetitive (rewarding) and aversive (punishing) events. Most of these paradigms are of associative nature and can be assigned either to classical or to instrumental conditioning. However, aversive memories might be formed also in non-associative manner, e.g. by sensitisation (for a detailed description of theoretical and practical aspects of the learning paradigms see Mackintosh 1974; Dickinson 1980; Dudai 1989; Eichenbaum and Cohen 2001).

For human beings, two different memory categories have been introduced. According to Schacter, implicit (or unconscious/unaware) memory is revealed when previous experiences facilitate performance on a task that does not require conscious or intentional recollection of those experiences. Explicit memory, in turn, is revealed when the performance of a task requires conscious recollection of previous experiences. These are descriptive concepts that are primarily concerned with a person's psychological experience at the time of memory retrieval. Accordingly, the concepts of implicit and explicit memory neither refer to nor imply the existence of two independent or separate memory systems (Schacter 1987). As these two memory categories cannot be easily applied to the situation in animals, they will not be further considered in this chapter.

2.1

Classical Conditioning

Classical ('Pavlovian') conditioning is a process whereby a subject learns the associative relationships between discrete elemental or configural stimuli, with

one stimulus being initially 'neutral' (or innocuous) to the animals (conditioned stimulus, CS) and the other (unconditioned stimulus, UCS) being able to evoke an unconditioned response. A distinct CS (designated CS+) comes to gain control over eliciting a conditioned response if the probability of a UCS occurrence in combination with the CS exceeds that of its unsignalled occurrence. If the two probabilities are equal, the CS has apparently no predictive value, in which case the lack of predictability itself is learned ('learned irrelevance'). If the probability of a UCS occurrence alone exceeds that of its combination with the CS, the CS (conditioned inhibitor, CS-) predicts the omission of the UCS. In latent inhibition studies, the CS will be presented several times before its pairing with a rewarding or aversive stimulus, with the consequence that animals will show a diminished conditioned response to it. Explicit unpairing of CS and UCS is often used as a control for the specificity of learning-induced changes in interneuronal communication, as both paired and unpaired protocols share similarities in number and intensity of CS/UCS presentation and differ solely in the temporal relationship between the two stimuli. However, this kind of control could be inappropriate, as unpairing induces a learning process as well, in that animals will regard the CS as a conditioned inhibitor.

The conditioned response elicited by a CS+ might be similar to the unconditioned response to the UCS. However, it seems to be more appropriate to assume that the conditioned response is elicited by the anticipation of the UCS rather than necessarily consisting of any component of the unconditioned response (Fanselow 1994; Gray and McNaughton 2000). The nature of the conditioned response depends on the UCS and the behavioural repertoire of a distinct species and cannot be controlled by the experimenter.

Fear conditioning and eyelid conditioning are the most frequently used paradigms of aversive classical conditioning. In these tasks, a tone or light (CS+) will be associated with a mild electric shock (UCS) applied either to the feet (fear conditioning) or to the eye (eyelid conditioning). As a consequence of this pairing, the CS+ will elicit a fear reaction that can be measured at the behavioural level as freezing (immobility except for breathing-related movements), fear-potentiated startle (potentiation of a normal startle reaction to a loud tone during presentation of the CS+, typically a light signal), conditioned suppression of an operant behaviour (e.g. lever pressing for food or water) or reflexive closure of the eye (eyelid or eye-blink conditioning). The conditioned emotional response becomes manifest also at the hormonal (increased secretion of stress hormones) and autonomic (e.g. tachycardia, galvanic skin response, rise in blood pressure) levels (Davis 2000). The majority of studies analyse the animals' freezing response to the CS. Other than measurements of startle responses or eyelid closures, this analysis does not require a sophisticated technical apparatus and enables behavioural observations in free, non-restrained animals. In any case, detailed knowledge about the neural basis of a selected behavioural response turns out to be essential for correct interpretation of the data in respect to the strength and persistence of the aversive

memory. For instance, electrical stimulation of the sensory pathway that relays information of the tone signal to the lateral amygdala triggers a sequence of different fear reactions ranging from increased vigilance via freezing to escape behaviours, depending on the intensity of stimulation (Lamprea et al. 2002). Under these circumstances, an extraordinarily strong association between tone and shock can result in panic-like behaviour rather than a pronounced freezing response. Uncritical reduction of a complex behavioural phenotype to a single behavioural parameter could, therefore, easily lead to false-negative or false-positive findings.

Fear conditioning depends on the temporal overlap of CS and UCS (contiguity). In a common conditioning protocol, the onset of the tone precedes the shock by several seconds and co-terminates with it (delay conditioning). In other cases (trace conditioning) there is an interval between the end of the tone and application of the footshock that can last from milliseconds to several seconds. On longer intervals, the CS will usually not be associated with the UCS anymore. The situation is different for conditioned taste aversion (see also Sect. 2.2), for which CS and UCS presentation can be separated by several hours (for review, see Welzl et al. 2001). In general, contingency of a particular CS (i.e. its ability to predict the occurrence of the UCS) seems to be more critical for memory acquisition than contiguity (Mackintosh 1983; Rescorla 1988).

Animals form associations not only between a discrete elemental or unimodal CS (i.e. tone, light or odour) and the UCS, but also between the more complex test situation (configural or polymodal CS) and the shock. Configural CS are composed of a complex 'meshwork' of different unimodal CS (such as the shape, structure, material and smell of the conditioning environment), of the handling procedure and of information about the inner state of the animals. Memory of elemental CS is called cued memory; memory of the configural CS, contextual memory. In a common auditory fear-conditioning task, the appearance of the tone is temporally connected tightly with the presentation of foot shock (foreground conditioning), whereas the conditioning context is more latent (background conditioning).

2.2

Instrumental Conditioning

Instrumental ('Thorndikian', operant) conditioning is the process whereby the animals acquire new behavioural patterns that enable them to alter the frequency of their exposure to stimulus events. Whereas in classical conditioning subjects learn about relations between signal and significant events such as food or danger (stimulus–stimulus association), in instrumental conditioning they learn about relations between their behaviour and those significant events (response–stimulus learning). In instrumental conditioning, the experimenter controls the occurrence of the stimuli. The animals, by contrast, have more control over the occurrence of the response. If the occurrence of the stimulus is

completely independent of the occurrence of responding, animals either do not change their baseline response rate (in case of a rewarding stimulus: learned irrelevance) or develop a special type of learned irrelevance called 'learned helplessness' (in case of aversive stimuli). If the probability that a response is followed by a rewarding stimulus (also called a positive reinforcer) is above chance levels, animals will show the behavioural response more frequently than during baseline conditions in order to maximise reward. Contrarily, if the response is followed by a punishment (also called a negative reinforcer), animals will reduce their response below baseline performance in order to minimise punishment. The latter situation is typical for passive (or inhibitory) avoidance paradigms in which animals are in an approach-avoidance conflict that performance of a natural behavioural response would lead to a punishment. Animals can avoid this punishment only if they remain passive. Typical examples for passive avoidance paradigms are step-down avoidance, step-through avoidance and conditioned taste aversion for rats and mice as well as bead pecking for chicks. In the step-down task, animals will be placed on a neutral platform that is localised on a metal grid. Animals receive a mild electric footshock as soon as they leave the platform. In the step-through task, animals will be placed onto the brightly illuminated floor of a test box that consists of a lit and a dark compartment connected by a sliding door. Because of their innate aversion to brightly lit environments, the animals will 'escape' to the dark compartment where they will receive a footshock. Memory performance is generally assessed by measuring the time until animals step down from the platform or leave the lit compartment on re-exposure to the respective test situation. In conditioned taste aversion (which contains aspects of both classical and instrumental conditioning), thirsty animals will be exposed to a fluid of novel taste (commonly a sucrose solution), followed by an injection of lithium chloride that causes nausea and discomfort. Animals will avoid consuming this fluid in the future, which is taken as a measure of the aversive memory. With bead-pecking avoidance, a similar task has been established for chicks. In this task, coloured beads are coated with a distasteful chemical compound and exposed to day-old chicks. Chicks that peck such beads show a disgust reaction and will avoid a similarly coloured but dry food in the future.

If in instrumental conditioning a response is not followed by a punishment, but its absence, animals will increase this response in order to minimise punishment. In active avoidance tasks, for instance, animals have to show a distinct behavioural response in order to avoid a punishment. Typical examples would be shuttle-box experiments and jump-up avoidance. A shuttle box consists of two compartments that are connected by a sliding door. The punishment will be signalled by either a tone or a light stimulus (CS). Animals have to leave the compartment in which the CS was presented within a selected amount of time, after which the CS would be followed by a footshock. In the pole-jump test, the occurrence of the footshock will be signalled by a tone or light stimulus as well. Animals can avoid the punishment if they jump onto a vertical wooden rod.

Memory performance will be assessed by the number of anticipatory responses (i.e. escape reactions during CS presentation). Whereas fear conditioning and passive avoidance tasks can be acquired within a single trial, active avoidance learning usually requires more intensive training. In case of repeated training, the distribution of learning events into several sessions (spaced learning) results in stronger memories than equivalent amounts of training crammed into a single session (massed learning).

2.3

Sensitisation

Both classical and instrumental conditioning are based on associative learning processes. However, animals might show an intensified or reduced behavioural response following non-associative learning as well. During sensitisation, a stressful, aversive event (e.g. footshock) leads to an unspecific increase in the sensitivity/reactivity to distinct sensory stimuli (Rosen and Schulkin 1998; Stam et al. 2000). The resulting aversive memory intensifies the animals' innate defence reaction. This definition implies that animals only become more sensitive to sensory stimuli that are generally able to elicit defensive reactions.

3

Animal Models

The majority of cellular signalling cascades involved in memory processing have been described in invertebrates, namely in the giant marine snail *Aplysia californica* (Abel and Kandel 1998; Kandel 2001) and the fruit fly *Drosophila melanogaster* (Dubnau and Tully 1998). Moreover, basic principles of memory consolidation have been discovered in chicks (Rose and Stewart 1999; Rose 2000). Nevertheless, this chapter will largely concentrate on rats and mice, which are the preferred experimental subjects for the study of cellular correlates of aversive learning and memory in mammals and most closely resemble neural processes of human being (Denny and Justice 2000; Bucan and Abel 2002).

Today, there are hundreds of different rat and mouse lines available. Newcomers to the field of animal experimentation might wonder what species and strains to use for a selected experimental question. Rats have the clear advantage over mice in that they are bigger (in particular when it comes to the stereotaxic targeting of small brain structures), less impulsive and superior to mice pertaining to the complexity of their behavioural 'repertoire' (Whishaw et al. 2001). Mice, in contrast, are the preferred subjects of geneticist. Their genome has been sequenced, and genetical tools for specific and sophisticated manipulations of the genome have been established exclusively for this species.

Moreover, their housing is less space- and cost-intensive, which predestines them for large mutagenesis screens, selective breeding and quantitative trait loci studies. Although rats are principally indispensable for behavioural experiments, mice will clearly dominate the experimental analysis of learning and memory for the next decade.

Before selecting one of the different mouse and rat strains available from commercial suppliers for a given experiment, one has to carefully consider the rationale of the planned study (Andrews 1996; Crawley et al. 1997; Owen et al. 1997). Mice from C57BL/6 strains, for instance, are good learners in a variety of memory tasks, including amygdala- and hippocampus-dependent conditioning. DBA/2 mice, by contrast, are poor learners in hippocampus-dependent paradigms, including contextual fear conditioning (Paylor et al. 1994; Gerlai 1998). The selection of C57BL/6 or DBA/2 strains would therefore depend on whether one expects impairment or amelioration of memory performance after a certain pharmacological treatment.

With the advent of modern mouse genetics, mice could be generated that bear either a transgene, which will be expressed under control of a specific promoter (transgenic mice), a specific point mutation in a given protein, a null mutation of a gene (conventional 'knock-outs') or ablation of a gene in temporally and locally restricted manner (conditional 'knock-outs') (Picciotto and Wickman 1998). The most advanced generation of mutant mice (inducible 'knock-outs') allows the timed inactivation of a given gene by pharmacological means (Mayford and Kandel 1999). The latter animals turn out to be extremely useful for analysis of the involvement of the respective gene product in different phases of the learning curve (e.g. Shimizu et al. 2000; Genoux et al. 2002; Kida et al. 2002). Unlike conventional and conditional 'knock-outs', these animals do not bear the risk that alterations in their memory performance are due to developmental defects or compensatory processes (Gingrich and Hen 2000; Gross et al. 2002). Strictly taken, studies performed in conventional 'knock-outs' investigate the animals' ability to cope with the life-long and ubiquitous ablation of a given gene product. Quite often, this ability depends on the genetic background of the animals, indicating that the mutation targeted a 'specific gene ensemble' rather than a single gene (Routtenberg 2002). In any case, it is strongly recommended to validate major findings obtained with mutant mice by comparing them with intact control animals using pharmacological means.

Embryonic stem cells for the generation of 'knock-out' mice have been available from a small subset of inbred strains only (i.e. 129 strains), which, ironically enough, turned out to be poor learners in a variety of learning and memory tasks (Montkowski et al. 1997; Cook et al. 2002). Experimental success, thus, largely depends on an optimal breeding strategy (Wolfer et al. 2002), which, however, requires the co-ordination between molecular biologists and behavioural scientist at early stages of mouse generation. As soon as two different mouse strains are mixed, we face the problem of genetic background, in particular for the generation of null mutants by homologous recombination

(Gerlai 1996, 2001). In such cases, animals have to be crossed with a pure inbred strain (commonly C57BL/6J lines) for at least 6–8 generations (backcrossing). For correct interpretation of data obtained in genetically modified mice it is indispensable to follow the strict rules of strain nomenclature (Wotjak 2003).

The breeding scheme of 'knock-out' mice defines not only the genetic background, but also the availability of control animals. Control animals (wild-type) bear no mutation on their chromosomes. Their behaviour will be compared with that of mice with a mutation on each allele (homozygous) or one allele only (heterozygous). For behavioural experiments, animals of all three genotypes should derive from the same heterozygous breeding pairs. As littermates, they share a similar life history with respect to maternal care and stress. Homozygous breeding pairs should be avoided, as the mutation might affect maternal behaviour, which, in turn, has strong influences on stress susceptibility and memory capabilities of the offspring (Meaney 2001). However, even for heterozygous breeding pairs, there is still a risk of observing false-positive differences between homozygous null-mutants and their wild-type littermate controls. The offspring are not passively nursed by their mothers, but interact with them and compete among each other for resources. As mutants are commonly weaker than their wild-type littermates, this situation might be of disadvantage for them and lead to long-lasting changes in their emotionality.

Housing conditions are another important factor that influences memory performance (Würbel 2001). In standard laboratory conditions, rats and mice are housed under sensory deprivation in an extremely impoverished environment. It is, therefore, not surprising that animals that grew up in an enriched environment are better learners (van Praag et al. 2000). However, enrichment might eventually 'overwrite' effects of a mutation (Rampon et al. 2000). Furthermore, it might increase the variability among the experimental subjects, with negative consequences for the statistical interpretation of the data.

4

Neurological Substrates of Aversive Memories

For a long time, scientists had been sceptical that memory could ever be assigned to specific brain regions. However, as distinct mental functions such as movement co-ordination, perception, attention and language could be localised to different regions, it turned out that memory processes also critically depend on selected brain structures (Milner et al. 1998). As learned behaviour can be regarded as a refinement and further development of intrinsic (innate or inherited) behaviour (Vanderwolf and Cain 1994), learning-induced alterations in interneuronal communication primarily occur in those brain circuits that are involved in expression of the respective behavioural response. These brain circuits might differ in a number of brain structures, depending on the characteristics of the stimuli processed (e.g. olfactory vs auditory fear con-

ditioning vs conditioned taste aversion). Nevertheless, there are a few brain structures that seem to be of general importance for most types of aversive learning. These structures include the amygdala (crucial for consolidation and, possibly, also storage of aversive memories; McGaugh 2000; LeDoux 2000) and the hippocampus (critical for learning and memory tasks in which discontiguous items must be associated, in terms of their temporal or spatial positioning; Wallenstein et al. 1998). Before I come to a short description of anatomical and functional features of these brain structures, I will briefly consider the methodological approaches that have led to the characterisation of such structures' importance for aversive memories.

4.1

How to Find a Candidate Brain Structure

First indicators for an involvement of a given brain structure in learning and memory processes are local changes in neuronal activity. These changes can be measured during different phases of the learning curve. In vivo methods [such as functional magnetic resonance imaging (fMRI), microdialysis procedures or electrophysiological recordings of electroencephalograms (EEG), sensory evoked field potentials and single unit activity] enable the monitoring of neuronal activity in conscious animals during memory performance. With these techniques, dynamic changes in neuronal activity can be observed over several learning phases within the same experimental subject. A disadvantage is, however, that most of these methods are cost intensive, invasive, show relatively poor temporal and spatial resolution (e.g. fMRI, EEG) and allow the simultaneous monitoring of a relatively small number of brain structures only.

In vitro (in situ) methods monitor neuronal activity off-line. For this purpose, animals have to be killed at a given time point of the learning curve, and markers of neuronal activity (e.g. expression patterns of immediate early genes or local accumulation of specific metabolic markers such as 2-deoxyglucose) (Sharp et al. 1993; Herdegen and Leah 1998; Sokoloff 2000) are visualised in the dissected brain. In vitro methods are less cost-intensive and less technically demanding than in vivo approaches. They allow, furthermore, analysis of a high number of brain structures. Elaborate statistical tools enable the characterisation of functionally relevant neuronal circuits (e.g. McIntosh and Gonzalez-Lima 1994). A disadvantage is, however, that these methods provide only a snapshot of neuronal activity during a distinct phase of the learning curve.

4.2

How to Prove a Causal Involvement in Learning Processes

Changed neuronal activity during a distinct learning phase provides at best an indirect hint for a critical involvement of this brain structure in memory

processes. Lesion studies are, therefore, indispensable as a proof of causalities. We distinguish between permanent and transient lesions. Permanent lesions can be achieved by electrocoagulation, aspiration, knife cuts or, preferably, local administration of excitotoxins (Jarrad 2001, 2002). Permanent lesions have generally the disadvantage that they cannot be confined to distinct phases of the learning curve. Transient lesions, by contrast, can be achieved by cooling of distinct brain structures or local administration of anaesthetics, tetrodotoxin (which blocks the propagation of action potentials) and muscimol [an agonist of the γ -aminobutyric acid (GABA)_A receptor]. Transient lesions allow for the dissection of the role of a brain structure for a given learning phase and provide information not only on 'where' but also 'when' and for 'how long' these processes take place, thus adding the chronological dimension to the topographical one (Ambrogio Lorenzini et al. 1999). The causal involvement of a distinct brain structure in learning and memory can, furthermore, be assessed by local pharmacological treatments. Compared to lesioning, this approach has not only the advantage that it is not destructive, but it is also informative as to the mechanisms of memory processing (Izquierdo and Medina 1998; McGaugh and Izquierdo 2000).

Brain structures can be lesioned either before (anterograde) or after (retrograde) a distinct phase of the learning curve. Notably, for anterograde lesioning there is the risk that animals bypass the lesioned brain structure and still show relatively normal memory performance, although the brain structure would have been involved in intact animals. In any case, care has to be taken that lesions or pharmacological treatments do not interfere with general locomotion, motivation or processing of sensory information, but specifically with memory processes.

4.3

Candidate Brain Structures

A variety of brain structures seem to be essential for aversive memories. In the following, I will briefly introduce the hippocampus and amygdala involvement, without disregarding the importance, for instance, of the cerebellum for eye-blink conditioning (Thompson et al. 1997, 2000; Medina et al. 2002) and the insular cortex for conditioned taste aversion (e.g. Berman and Dudai 2001).

4.3.1

Hippocampus

The hippocampus received its name from the similarity of the human hippocampus to the tail of a seahorse (Latin name, hippocampus). In mice and rats, however, there is little resemblance to a seahorse. In fact, in these species the hippocampus has a rather 'banana-like' shape in its rostral-caudal extension. Morphologically and functionally, scientists differentiate between the

dorsal pole (also septal pole because of its close connections with the septum) and the ventral pole of the hippocampus. The hippocampus contains several anatomically and functionally well-defined cell fields (CA1 to CA4, named after *Cornu ammonis*, a snail that stimulated the morphologists' imagination in a similar manner as the seahorse did when it came to the description of the human hippocampus). Together with the entorhinal cortex, the dentate gyrus and the subiculum, the hippocampus comprises the hippocampal formation (Amaral and Witter 1989).

Inputs to the hippocampus are spread to the different cell fields primarily by the famous trisynaptic pathway (Amaral and Witter 1989). According to this simplified circuit, the entorhinal cortex projects to the dentate gyrus via the perforant path, the dentate gyrus to CA3 region via the mossy fibres and the CA3 region to the CA1 region via the Schaffer collateral. This trisynaptic pathway turned out to represent an excellent model system for studying cellular processes of synaptic plasticity. The fact that this pathway remains intact in coronal sections of the rat and mouse brain and that the subfields can be easily visualised opened the avenue for studying synaptic plasticity under in vitro conditions.

Plenty of evidence suggests an essential role for the hippocampus in the formation and extinction of aversive memories, in particular in passive avoidance learning (Izquierdo and Medina 1997). The hippocampus is not essential for acquisition and recall of cued fear memories in delay fear conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992). In contrast, it plays an important role for the processing of aversive memories following trace fear conditioning (Berger and Thompson 1976; McEchron et al. 1998) and background contextual conditioning tasks (Maren and Holt 2000; Sanders et al. 2003; but see also Gewirtz et al. 2000). As background contextual conditioning occurs in parallel to the acquisition of cued fear memories, analysis of drug effects on each of the two components enables the dissection of hippocampus-dependent from hippocampus-independent memory processes and unspecific effects of a pharmacological treatment or mutation (e.g. on locomotion, emotionality, general sensitivity to sensory inputs).

4.3.2

Amygdala

The amygdala is the most prominent brain structure pertaining to the generation of negative emotions, including fear and anxiety (LeDoux 2000; Adolphs 2002; Dolan 2002). It has been named after its structural similarities in humans with an almond (Latin name, amygdala). The amygdala is a heterogeneous collection of interconnected nuclei in the depth of the temporal lobe that differ morphologically and functionally. A detailed description of its complex structural organisation and functions is given elsewhere (Swanson and Petrovich 1998; Pitkänen 2000). In brief, the amygdala contains cortical and striatal com-

ponents. The cortical components (i.e. the basolateral amygdala complex that combines lateral, basolateral and basal amygdala) seem to be essential for both cued and contextual fear conditioning, in particular for the association between CS and UCS. Efferents of the basolateral amygdala to extra-amygdaloid brain structures are thought to regulate active responses to potentially dangerous stimuli or situations (Killcross et al. 1997). The striatal components comprise the medial and central nuclei, the latter of which receives inputs from the basolateral amygdala complex and orchestrates the defensive reactions of the animals to the aversive stimulus events. The role of the basolateral amygdala complex as a place where aversive memories are not only acquired, but also consolidated and stored, is disputed (Cahill et al. 1999; Fanselow and LeDoux 1999). According to the consolidation hypothesis (McGaugh 2000), the basolateral amygdala complex facilitates the consolidation of aversive memories in other brain structures, but does not serve as a storage site itself. In any case, the amygdala (in particular the basolateral amygdala complex) is essential for memory acquisition and consolidation in passive avoidance tasks and fear conditioning (delay and trace cued conditioning, contextual conditioning) (LeDoux 2000; Maren 2001).

Sensory information about auditory stimuli reach the lateral amygdala via two different pathways, either directly from thalamic relay structures, such as the medial geniculate nucleus, or from cortical structures (auditory cortex). Information transfer via the thalamus is faster but less precise when it comes to the exact recognition of the sensory stimulus. In contrast, information processed by cortical structures is precise but needs longer to reach the amygdala (LeDoux 1998, 2000). An ultimate explanation of this parallel processing of sensory stimuli might be that it seems less devastating for an animal to react immediately to a potentially harmful stimulus with a false alarm, than to react to it too late (LeDoux 1996).

5

Cellular Mechanisms Underlying Aversive Learning and Memory

More than a century ago, Ramón y Cajal and Tanzi postulated that cellular mechanisms of learning and memory include both the formation of new synaptic connections and the restructuring of the existing ones to make the interneuronal communication more efficacious (for review and references see Geinisman 2000). Another 50 years later, Donald Hebb formulated his famous principles of memory encoding, stating that “When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased” (Hebb 1949; for review see Sejnowski 1999). Today, it is generally accepted that learning leads to transient or permanent modifications in interneuronal communication via morphological or functional changes of synaptic contacts (Milner et al. 1998;

Woolf 1998; Geinisman 2000). A variety of cellular models of learning and memory have been established, including long-term potentiation (LTP), long-term depression (LTD; for comprehensive review see Martin et al. 2000) and kindling (Adamec and Young 2000; Hannesson and Corcoran 2000). LTP and kindling are induced by repetitive high-frequency stimulation of discrete brain areas or specific pathways and characterised by long-lasting hyperexcitability to single electrical pulses. LTD, in turn, is induced by low-frequency stimulation and stands for decreases in neural excitability. Despite the ongoing debate about their physiological significance (e.g. Hölscher 1997; McEachern and Shaw 1999), LTP and LTD have a high number of cellular processes in common with learning and memory (Martin et al. 2000; Blair et al. 2001; Brauneuwel and Manahan-Vaughan 2001; Goossens and Maren 2002).

5.1

Memory Acquisition

There is good evidence that both associative and non-associative learning lead to a strengthening of synaptic contacts. For instance, in auditory cued fear conditioning, coincident depolarisation of principal (i.e. glutamatergic pyramidal) neurones within the lateral amygdala by a tone and a footshock results in a potentiation of those synapses, which relay the auditory information to that brain structure (Rogan et al. 1997; Collins and Paré 2000; Tang et al. 2001). This potentiation becomes evident by an increase in evoked field potentials compared to baseline responses. However, as field potentials integrate over the activity of multiple neurones and might even be volume conducted from other brain structures, care has to be taken that the changes in interneuronal communication indeed originate from the lateral amygdala. Studies verified the lateral amygdala as the place of learning-induced changes in synaptic transmission by local infusion of anaesthetics (Tang et al. 2003) as well as by single-unit (e.g. Collins and Paré 2000) and intracellular recordings (Rosenkranz and Grace 2002). The potentiation of synaptic transmission may affect each of the two principal inputs to the lateral amygdala, the thalamic (Rogan et al. 1997) and the cortical pathways (Tsvetkov et al. 2002). The similarities of memory acquisition and LTP induction include cooperativity (a neurone must reach a threshold of depolarisation before learning-induced or LTP-induced synaptic changes can occur) and associativity (pairing stimulation of a weak pathway with stimulation of a strong pathway results in facilitated synaptic transmission in both pathways). Both memory acquisition and LTP induction depend on a special form of ionotropic glutamate receptors [*N*-methyl-D-aspartate (NMDA) receptors], protein kinases, voltage-gated calcium channels and protein synthesis (e.g. Schafe et al. 2001; Blair et al. 2001). Recently, with the gastrin-releasing peptide, a transmitter could be described that is specifically expressed in the brain circuit responsible for fear conditioning and involved in both induction of LTP in the cortical afferents to the

lateral amygdala and auditory-cued fear conditioning (Shumyatsky et al. 2002). Other studies revealed that learning-induced changes in synapses of the cortical projections are under negative control of the medial prefrontal cortex (Grace and Rosenkranz 2002). In this context, dopamine seems to play a crucial role for memory acquisition, as it overrides the inhibitory input from the medial prefrontal cortex and potentiates the cortical input.

The amygdala is not the only brain structure where learning causes a rebuilding of synaptic contacts during aversive learning. Sensitisation by application of an electrical footshock, for instance, affects synaptic transmission in the septal-hippocampal system (Thomas 1988; Garcia 2002). Most prominent, however, are the refinements of receptive fields in the auditory cortex. On more intensive training protocols than the few tone-shock pairings usually applied in fear conditioning paradigms, neurones of the auditory cortex become sensitive to the frequency of the tone used during conditioning (Weinberger 1998; Edeline 1999). Importantly, these changes are not restricted to classical conditioning, but are also evident following active avoidance learning. They include modifications that allow gerbils (a species with excellent hearing capabilities) to form categories about special features of more complex tone signals (Ohl et al. 2001).

Auditory-cued fear conditioning leads to a potentiation of field potentials not only within the lateral amygdala but also within the hippocampus (Doyere et al. 1993; Tang et al. 2003). On first glance, this observation has been astonishing, as the hippocampus is not essential for acquisition and recall of aversive memory to the tone during delay conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992). However, a recent publication provides the first evidence for its physiological relevance (Moita et al. 2003) that might only become evident in more complex test situations (Doyere et al. 1993).

Only a few studies used *in vivo* electrophysiological recordings for contextual conditioning or passive avoidance learning (e.g. Sacchetti et al. 2001). In contrast, cellular mechanisms underlying memory acquisition in these tasks were extensively studied by pharmacological and genetical means (Izquierdo and Medina 1997; Schafe et al. 2001; Silva 2003). Similarities between induction of hippocampal LTP and memory acquisition are evident. However, a causal relationship between these two processes still remains to be shown (Gerlai 2002).

With the technical progress being made in molecular biology, it has become possible to screen for genes that might be critically involved in acquisition (and consolidation) of aversive memories. These techniques include mutagenesis screens and quantitative trait loci studies. In large mutagenesis screens, breeding pairs are treated, for instance, with the highly mutagenic compound *N*-ethyl-*N*-nitrosourea (ENU) (Anderson 2000; Brown and Balling 2001). Offspring of these breeding pairs are tested for their memory capabilities. The quantitative trait loci approach, in contrast, is based on the different behavioural performance of genetically heterogeneous mice. In

a typical experimental situation, animals of two different inbred strains are crossed, with the consequence that the F1 generation shares 50% homology of their genome with each of its parental strains. F1 animals are then crossed with mice from their parental strains, with the consequence that animals of the F2 generation are genetically and behaviourally heterogeneous due to homologue recombination during meiosis (crossing-over). Animals of the F2 generation are ranked according to their behavioural performance in the learning task. For the upper and the lower 10%, the contribution of genes from the two parental strains to the behavioural phenotype are estimated using polymorphism markers (Wehner et al. 2001).

5.2

Memory Consolidation

More than a century ago, Müller and Pilzecker proposed the perseveration-consolidation hypothesis, according to which new memories initially persist in a fragile state and consolidate over time to reach a state in which they are insensitive to disruption (Lechner et al. 1999 and references therein). About 50 years later, Gerad and Hebb independently from each other came up with the dual-trace theories of memory, suggesting that short-term and long-term memories are sequentially linked, and stabilisation of reverberating neural activity (underlying short-term memory, lasting for seconds to hours) produces long-term memory (lasting for hours to months) (McGaugh 2000 and references therein). Later on, however, it could be demonstrated that drugs might selectively block either short-term or long-term memory, indicating that these two processes occur independently and in parallel (McGaugh 2000; Izquierdo et al. 2002).

Memory consolidation becomes manifest in morphological and functional changes of synaptic contacts. The underlying cellular mechanisms have been studied by pharmacological manipulation, activity monitoring and genetic approaches (for review see Martin et al. 2000; Kandel 2001; Silva 2003). In this way, two different groups of agents could be characterised: permissive agents and instructive agents. Permissive agents may 'arouse' brain structures. They are necessary, since they aid the instructive agents, but are not sufficient for memory storage. Instructive agents, by contrast, directly modify synaptic strength (Shobe 2002), for instance by directly altering transmitter release, by receptor sensitisation/desensitisation and by structural rearrangements. Whereas permissive agents are rather ubiquitously distributed throughout a neurone following memory acquisition, instructive agents are confined to those synaptic terminals that undergo functional and/or morphological changes during learning. The mechanisms underlying this synapse specificity still remain elusive. However, with the synaptic tag hypothesis there is a promising concept for future investigations (Frey and Morris 1998). According to this hypothesis, consolidation at local sites represents a dual process: memory acquisition induces both cell-wide expression of macromolecules and the formation of local

postsynaptic 'tags' that 'hijack' only the macromolecules to those synapses that are involved in the memory engram.

Changes of neural processes initiated by memory acquisition follow different time courses for different brain structures. For instance, transient inactivation of the basolateral amygdala interfered with consolidation of cued and contextual memory for 48 h, whereas the perirhinal cortex was sensitive to retrograde amnesia for 192 h (Sacchetti et al. 1999). Long-term consolidation of contextual fear (Shimizu et al. 2000) and spatial memory (Riedel et al. 1999) requires recurrent activation of hippocampal ionotropic glutamate receptors for about 1 week following conditioning, which led to the synaptic re-entry reinforcement hypothesis of memory consolidation (Wittenberg and Tsien 2002). Obviously, it seems to be an evolutionary advantage to delay memory consolidation until the significance of an experience could be evaluated. In fact, events that precede or follow memory acquisition are able to interrupt the consolidation process by proactive and retroactive inhibition (Xu et al. 1998; Izquierdo et al. 1999), possibly via LTD-like mechanisms (Manahan-Vaughan and Braunewell 1999). Accordingly, there seem to be similar critical phases for consolidation of LTP, during which a depotentiation is possible (Huang and Hsu 2001; Lin et al. 2003a).

Consolidation processes for short-term and long-term memories are distinguished by their dependency on *de novo* protein synthesis (Davis and Squire 1984; Matthies 1989; Izquierdo et al. 2002). Blockade of transcription or translation by drug infusion into lateral amygdala or hippocampus revealed that the consolidation of long-term but not short-term memories for cued and contextual fear conditioning as well as passive avoidance learning required protein synthesis (Schafe and LeDoux 2000; Kida et al. 2002; Muller Igaz et al. 2002). Interestingly, there seem to be at least two different waves of protein synthesis necessary for memory consolidation, with peaks between 0–1 h and 3–6 h after conditioning (Bourtchouladze et al. 1998; Muller Igaz et al. 2002), corresponding to those described for bead pecking in chicks (Freeman et al. 1995). Protein synthesis will be initiated via a cascade of second messenger systems and protein kinases that, in turn, activate transcription factors [such as the cAMP-responsive element binding protein (CREB)] and finally transcription (Milner et al. 1998; Clayton 2000; Kandel 2001). The activity of this consolidation cascade is negatively controlled at various levels, for instance by protein phosphatases and repressors of transcriptional activity (Abel and Kandel 1998; Cardin and Abel 1999; Kandel 2001; Genoux et al. 2002).

The characterisation of memory-related genes and proteins belongs to the hot spots of current memory research (D'Agata and Cavallaro 2002). Respective studies in the field of aversive memories employ different molecular biological methods including *in situ* hybridisation (Ressler et al. 2002), differential display (Huang et al. 1998), subtractive hybridisation (Stork et al. 2001) and DNA microarrays (Kida et al. 2002). Most critical for the correct interpretation

of the data are the selections of appropriate controls, as unspecific changes in gene expression may occur already from handling of the animals and exposure to the test context. Another critical point is the dissection of biological material. So far, the methods employed, in particular for analysis of the proteome, require relatively high amounts of protein, with the consequence that whole brain structures have to be analysed. However, only a small subset of neurones or even synapses of a given neurone might be involved in a distinct memory process, and neurones are highly heterogeneous in their gene expression profiles (Kamme et al. 2003; Levisky and Singer 2003). Consequently, the signal-to-noise ratio would be very small on average over whole brain structures, making subtle changes in gene expression, expected for learning events, hard to detect (Geschwind 2000).

The role of the amygdala for consolidation of aversive memories is generally accepted. However, as stated before, it is still debated as to whether or not the amygdala is also the storage site for the consolidated memories. Some authors suggest that the amygdala is essential for memory consolidation and storage in other brain structures only. According to their hypothesis, punishment used for aversively motivated learning activates the two major hormonal stress systems of the organism, the hypothalamic–pituitary–adrenal axis (with corticotropin and corticosterone/cortisol) and the sympatho-adrenergic system (with noradrenaline and adrenaline). Both stress systems seem to funnel into the same regulatory system at the level of the amygdala. The resulting potentiation of the local effects of noradrenaline leads to an activation of efferent projections that are known to modulate plastic changes, for instance within the hippocampus (McGaugh and Roozendaal 2002).

Interestingly, the role of the hippocampus as a storage site of aversive memories is temporally limited. Contextual fear memories are susceptible to lesions of the hippocampus only for 3–4 weeks after memory acquisition (Kim and Fanselow 1992). During this time, they become finally consolidated in neocortical structures in a process that is called systems reconsolidation. Memories that have become independent of the hippocampus with time are referred to as ‘remote’ memories. Cellular correlates of systems reconsolidation are fairly unknown. It is conceivable that these processes involve reiteration of learning-induced changes in neuronal activity during waking and sleep (for reviews see Sejnowski and Destexhe 2000; Sutherland and McNaughton 2000; Graves et al. 2001; Paré et al. 2002) as well as principles of homeostatic plasticity (Turrigiano and Nelson 2000).

Another striking characteristic of aversive memories is their ability to reconsolidate on reactivation. By definition, memories should be insensitive to disruption, for instance by electroconvulsive shocks or drugs, once they have been consolidated. This is, in fact, the case as long as the treatments do not coincide with memory recall. Reactivation of a memory, however, makes it ‘labile’ again because of reconsolidation processes (Sara 2000; Nader 2003). Reconsolidation resembles consolidation in that similar cascades of molecular

events seem to be activated, including phosphorylation of the transcription factor CREB (Hall et al. 2001a; Kida et al. 2002), expression of immediate early genes (Hall et al. 2001a,b) and protein synthesis (Nader et al. 2000). However, reconsolidation occurs faster and is more sensitive to amnesic challenge than initial memory consolidation (Nader 2003). Even remote aversive memories return to a labile hippocampus-dependent state on reactivation. Reconsolidation processes initiated in this way are sensitive to the memory-disrupting effects of protein synthesis blockade again (Debiec et al. 2002). However, not all forms of remote aversive memories seem to undergo reconsolidation in a labile state (Milekic and Alberini 2002), an observation that deserves further investigation. Nevertheless, retrieval-induced reconsolidation might have evolved as a useful mechanism for dynamically integrating new information into pre-existing memory engrams.

5.3

Memory Retrieval

Memory retrieval is the only direct measure of memory in animal experiments. However, in the absence of controls that closely match the conditions for performance, it is difficult to make inferences about the role of a neurobiological process in retrieval. Depending on the circumstances, memory retrieval might lead to memory reconsolidation or memory extinction (Nader 2003). It is, therefore, not surprising that it shares cellular mechanisms, such as dependency on protein kinases and activation of immediate early genes (Hall et al. 2001a,b; Szapiro et al. 2002), with each of the two other processes. Interestingly, a recent study (Murchison et al. 2004) ascribes an important role in retrieval of aversive memories to noradrenaline, thereby challenging concepts about its primary involvement in consolidation processes (McGaugh and Roozendaal 2002).

5.4

Memory Retention

Memory retention and memory decay describe the same phenomenon but from two different perspectives: either we emphasise that memories are relatively stable over time (memory retention) or that they dissipate with time (decay, temporal degradation). So far, little is known about the cellular correlates of memory retention/decay. If memory acquisition and consolidation indeed lead to long-term changes in synaptic contacts and interneuronal communication, the question remains as to how these changes are maintained over long period of times, despite the regular protein turnover and hormonally mediated structural reorganisation of dendritic arbours. Recent data suggest that these processes include subunit-specific dynamic changes in the expression of distinct ionotropic glutamate receptors in the postsy-

naptic membrane (for review see Malinow and Malenka 2002). Other authors postulate 'mnemogenic' chemical reactions as the basis of memory retention, including phosphorylation/autophosphorylation and conformational changes (Roberson and Sweatt 1999). Failures of these processes or counterregulatory mechanisms (e.g. dephosphorylation) would, consequently, lead to memory decay (e.g. Genoux et al. 2002).

5.5

Memory Decay and Extinction

Everyone experiences how memories may dissipate with time. Responsible for this phenomenon might be spontaneous forgetting, the suppression of memory retrieval and memory extinction. Spontaneous forgetting describes the loss of learned performances that is often observed when time elapses between memory acquisition and memory retrieval. It results from different processes (Bouton et al. 1999). First, memory traces might dissipate over time. Second, information might increasingly interfere with retrieval of the original memory in a proactive or retroactive manner. Third, memory retrieval might be disturbed transiently or permanently (e.g. following head injury or stroke). Fourth, a recent study suggests that neurogenesis in the dentate gyrus might play a role in the clearance or destabilisation of outdated hippocampal memory traces after systems reconsolidation, thereby saving the hippocampus from overload (Feng et al. 2001). Furthermore, on confrontation with reminders of unpleasant or traumatic events, we often try to refocus attention and ignore the unwanted memory. The ability to suppress memory retrieval is accomplished, in part, by executive-control mechanisms as a special case of response-override situations (Levy and Anderson 2002).

It is a matter of debate whether memories can be erased at all (Jacobs and Nadel 1985). However, there is some evidence from animal experiments that processes that lead to learning or LTP-induced changes in interneuronal communication can be reversed by specific opponents of the consolidation cascade, as for instance phosphatases (Genoux et al. 2002; Lin et al. 2003a,b). The decay of both LTP and memory seems to depend on NMDA receptors. However, there are conflicting data as to whether recurrent activation of NMDA receptors promotes decay or long-term consolidation processes (Shimizu et al. 2000; Villarreal et al. 2002).

Whereas memory decay describes the actual loss of memory, the term extinction stands for an active learning process that suppresses rather than erases the original memory (Bouton and Swartzentruber 1991; Myers and Davis 2002). Extinction requires memory retrieval in the absence of positive or negative reinforcement. The conclusion that extinction can be regarded as an active process bases on the following observations: First, extinction retention dissipates over time thus resulting in re-occurrence of the extinguished conditioned response (spontaneous recovery). Second, the extinguished mem-

ory performance reappears in a context different from that used for extinction training (renewal). Third, presentation of the UCS following extinction training re-activates the extinguished CS–UCS association (memory reinstatement).

Extinction seems to engage the same brain structures as memory acquisition and consolidation (Myers and Davis 2002; Schwaerzel et al. 2002). A variety of neurotransmitter and second messenger systems could be characterised that contribute to extinction of aversive memories, including glutamate (via NMDA receptors), GABA, dopamine, noradrenaline (via β -adrenergic receptors), selected forms of voltage-gated calcium channels, protein kinases, phosphatases and endocannabinoids (for comprehensive review see Myers and Davis 2002). If extinction training indeed represents an active learning process during which the animals learn that the CS does not predict the occurrence of the UCS anymore, it would be interesting to analyse what transmitter systems are of particular importance for which phase of the extinction learning curve. So far, however, animals have mostly been treated before extinction training, thus leaving open whether the pharmacological intervention interfered with the initiation or consolidation of extinction.

As mentioned before, retrieval renders the consolidated memories labile again. It seems to depend on the test situation, whether this labile state is followed by reconsolidation or extinction of the aversive memory. Blockade of reconsolidation processes (e.g. by interrupting protein synthesis within the lateral amygdala), for instance, has led to extinction of the fear responses to the CS in an auditory fear-conditioning paradigm (Nader et al. 2000).

Several studies suggest that the prefrontal cortex plays an important role in retention of extinction by reducing amygdala-dependent fear reactions. On inappropriate functioning, animals show abnormal perseveration of fear responses (Garcia 2002). However, data about the involvement of the prefrontal cortex in fear conditioning are relatively inconsistent (Morgan and LeDoux 1995; Gewirtz et al. 1997), which might be related to the anatomical and functional heterogeneity of this brain area. In fact, lesions that spared with the caudal infralimbic nucleus a prominent part of the ventromedial prefrontal cortex had no effect on extinction (Gewirtz et al. 1997). By contrast, lesions that included this brain structure resulted in impaired extinction consolidation and retention (Quirk et al. 2000). Furthermore, infralimbic neurones showed an increased activity to the CS (i.e. tone) following extinction training that was negatively correlated with the animals' freezing reaction. Importantly, stimulation of infralimbic neurones by electrical impulses that resembled extinction-induced changes in neuronal activity simulated extinction memory in the absence of extinction training (Milad and Quirk 2002).

The medial division of the prefrontal cortex seems to contribute to memory extinction as well. It is under negative control of the basolateral amygdala (Pérez-Jaranay and Vives 1991; Garcia et al. 1999), most likely via activation

of the mesocortical dopamine system (Davis et al. 1994; Morrow et al. 1999; Tzschentke 2001). After complete extinction of the fear response, which coincides with a return to baseline of learning-induced potentiation of neural activity within the basolateral amygdala complex (Rogan et al. 1997; Tang et al. 2001), the medial prefrontal cortex will be activated and gain inhibitory control over the amygdala (Garcia 2002). The latter process likely involves the activation of inhibitory interneurons within the basolateral amygdala (Grace and Rosenkranz 2002).

The septal-hippocampal system is another prominent brain circuit involved in extinction of aversive memories. It is the major component of the behavioural inhibitory system of the brain and essential for the suppression of aversive emotional states (Thomas 1988; Gray and McNaughton 2000; Garcia 2002). For instance, long-term changes in septal-hippocampal efficacy turned out to be critical for the inhibition of behavioural despair, including the expression of freezing behaviour (Garcia 2002).

It is of importance to note that cellular mechanisms of extinction might vary for classical and instrumental conditioning paradigms. In classical conditioning tasks, the experimenter can control the rate of extinction by repeated presentation of the CS that cannot be avoided by the animals. In commonly used protocols of instrumental conditioning, by contrast, the animal decides when to start extinction, for instance by stepping down from a platform (step-down avoidance) or starting to consume the sucrose solution again (conditioned taste aversion). These procedural differences might well explain the inconsistencies in molecular correlates of extinction that have been reported for the two conditioning tasks (Myers and Davis 2002). Future studies on extinction of avoidance learning and conditioned taste aversion should, therefore, consider withholding the reinforcers of avoidance through blocking avoidance (response prevention) and prolonging the exposure to the CS/aversive situation (flooding, Baum 1973).

Extinction can be induced either by a few long-lasting presentations of the CS (massive extinction training) or by a series of short-lasting CS (graded extinction training). It critically depends on the training protocol, as the circumstances of memory retrieval seem to define whether the re-activated memory undergoes reconsolidation or extinction. Short-lasting presentations of the CS may cause a flashback of the aversive memory and, together with the aroused state of the animals, reconsolidation. Presentation of longer-lasting CS, in contrast, often goes along with an acute within-session extinction of the fear response that might be essential for consolidation and retention of extinction (Nader 2003). Moreover, the strength of the originally formed aversive memory determines whether memory retrieval leads to reconsolidation (strong memories) or extinction (weak memories) (Eisenberg et al. 2003). This has to be taken into account if extinction of aversive memories should be modulated by pharmacological means.

6

Clinical Implications

Most basic researchers and clinicians believe that detailed knowledge about cellular mechanisms underlying the formation and extinction of aversive memories will lead to the development of novel therapeutic strategies for the treatment of human anxiety disorders. Our current knowledge about these mechanisms largely arises from results obtained in animal experiments. However, the transferability of such experimental data to the human situation depends on the validity of the experimental models chosen. Numerous sets of criteria have been developed for evaluating experimental models (McKinney and Bunney 1969; Willner 1984; Newport et al. 2002). Human psychiatric disorders may develop as a consequence of genetic and developmental predisposition that affect sensitivity to life stress and the initiation of pathological processes. Consequently, it appears rather unlikely that comprehensive animal models can be developed that accurately reflect the human situation (Shekhar et al. 2001). These limitations apply also for fear conditioning and sensitisation (e.g. Yehuda and Antelman 1993) that are, nevertheless, frequently discussed as experimental models for pathological anxiety (i.e. phobias, generalised anxiety disorders, panic disorder and post-traumatic stress disorder) (Marks and Tobena 1990; Charney and Deutch 1996; Rosen and Schulkin 1998; Bouton et al. 2001; Garcia 2002). Rosen and Schulkin (1998) suggest that pathological anxiety evolves directly from normal fear responses. The pathology of these anxiety disorders would include hyperexcitability in the amygdala and the bed nucleus of the stria terminalis, caused by a process of neural sensitisation or kindling in which psychosocial stressors initiate changes in the fear circuits that lead to enhanced perception and response to subsequent threat and danger. On the other hand, one of the key functions of the amygdala might be the potentiation of vigilance by lowering neuronal thresholds in sensory systems. As a consequence, pathological anxiety may not be a disorder of fear, but a disorder of vigilance (Davis and Wahlen 2001). Both concepts are based on hyperexcitability of the amygdala, which can be experimentally induced by conditioning and sensitisation as well as LTP and kindling protocols. Hence, the respective animal and cellular models seem to be at least analogous (if not homologous) to the situation in patients and might, therefore, guide our search for novel therapeutic strategies for the treatment of pathological anxiety.

Data of animal experiments discussed in this chapter suggest a variety of potential pharmacological targets for the treatment of pathological anxiety (Fig. 1). As the occurrence of traumatic events is usually unpredictable, it seems more promising to interfere with consolidation than with acquisition processes. In this context, the sympatho-adrenergic and the hypothalamic-pituitary-adrenal system are of particular interest. Both noradrenaline and corticosterone/cortisol are known to facilitate memory consolidation, in par-

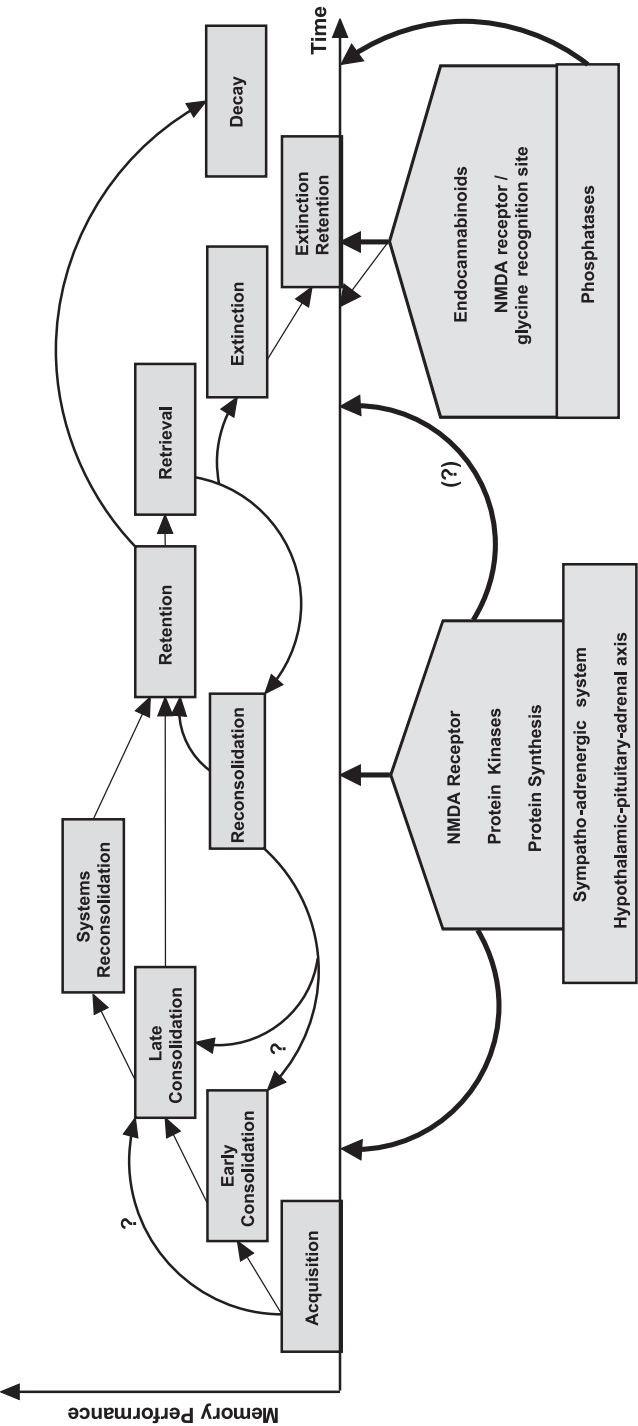


Fig. 1 Potential therapeutic targets for the treatment of pathological forms of aversive memories. The upper panel illustrates a typical learning curve. Subsequential phases are connected by arrows; still-uncertain interrelations are indicated by question marks. The lower panel shows some of the most promising targets for the pharmacotherapy of aversive memories. Consolidation, reconsolidation and extinction share a variety of cellular processes and, consequently, a common set of potential therapeutic targets. Other therapeutic targets are rather specific for memory extinction and decay. This schematic illustrates that pharmacotherapy has to be carefully adjusted to the different phases of the learning curve

ticular for aversive events (Cahill and McGaugh 1998; Korte 2001; McGaugh and Roozendaal 2002).

Treatments targeting these stress hormone systems would be restricted to a relatively narrow time window around the learning event. Quite often, however, patients might consult a physician long after the traumatic incident, when aversive memories have already been consolidated. Under these circumstances, the reconsolidation hypothesis gains particular importance, according to which (aversive) memories return to a labile state on retrieval. It appears to be possible to 'erase' aversive memories, if memory recall would be combined with a pharmacological treatment or electroconvulsive therapy (Sara 2000; Nader et al. 2000; Davis and Myers 2002). Depending on the protocol used, retrieval might initiate memory extinction rather than memory reconsolidation. This observation has already been used in the clinical praxis in form of exposure therapy (Marks and Tobena 1990), for instance for the treatment of post-traumatic stress disorder (Ballenger et al. 2000; Foa 2000; Rothbaum and Schwartz 2002). Exposure therapies are laborious for both patients and therapists. Data from animal experiments suggest that such therapies could become more efficient if they would be combined with pharmacotherapy (Myers and Davis 2002; Davis and Myers 2002) targeting, for instance, the glycine recognition site of the NMDA receptor (Walker et al. 2002), the endocannabinoid system of the brain (Marsicano et al. 2002) or protein kinases (Lu et al. 2001; Cohen 2002). Another potential target would be the GABAergic system, which, however, seems to be involved primarily in the expression of extinction that has already been acquired and not the extinction process per se (Davis and Myers 2002). Experiments performed with D-cycloserine, an agonist of the glycine recognition site of the NMDA receptor, demonstrate how efficiently animal studies on fear extinction (Walker et al. 2002; Ledgerwood et al. 2004; Richardson et al. 2004) can be transferred into the pharmacotherapy of human anxiety disorders (Ressler et al. 2004). Certainly, with the implementation of genomics, proteomics and pharmacogenomics in animal experiments on aversive memories, many novel therapeutical targets will be discovered for the benefit of patients. Exciting times!

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